



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4402PTWO/er	FOR FURTHER ACTION	See Form PCT/PEA/416
International application No. PCT/EP2004/051758	International filing date (<i>day/month/year</i>) 10.08.2004	Priority date (<i>day/month/year</i>) 12.08.2003
International Patent Classification (IPC) or national classification and IPC C12N5/00, C12N5/06, A61K35/12		
Applicant ISTITUTO NAZIONALE PER LE MALATTIE INFET... et al.		
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> <i>sent to the applicant and to the International Bureau</i> a total of 3 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>		
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input checked="" type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>		
Date of submission of the demand 10.06.2005	Date of completion of this report 09.08.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer van Heusden, M Telephone No. +49 89 2399-8145 <div style="text-align: right;">  </div>	

INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITYInternational application No.
PCT/EP2004/051758

IAP20 Rec'd PCT/FTO 10 FEB 2006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

Description, Pages

1-13 as originally filed

Claims, Numbers

1-30 received on 12.07.2005 with letter of 12.07.2005

Drawings, Sheets

1/1 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051758

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

see separate sheet

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	10,13,23-30
	No: Claims	1-9,11,12,14-22
Inventive step (IS)	Yes: Claims	.
	No: Claims	1-30
Industrial applicability (IA)	Yes: Claims	1-30
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)

PCT/EP2004/051758

Additional remarks to section II:

1. The documents mentioned in this report are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.
2. The priority document pertaining to the present application was not available at the time of establishing this report. Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the document by Bordoni (cited as D1 in the International Search Report) could become relevant to assess whether the subject matter of claims 1-29 of the present application satisfies the criteria set forth in Article 33(1) PCT.

Additional remarks to section V:

1. Novelty and inventive step (Article 33(2)(3) PCT)

- 1.1 The present application relates to a culture medium conditioned by immortalized, non-transformed and differentiated hepatocytes, e.g. MMH cells (met murine hepatocytes).

1.2 Interpretation of the claims:

Claims 1-11 relate to the culture medium itself (not to its use). Claim 1 states that the medium is free from conditioning cells when used for maintenance, proliferation and differentiation of adult mammalian cells. Thus claim relates to a medium that is free from conditioning cells, claim 1 does not relate to the use of said medium.

The subject matter of claims 4-6 refers to the intended use of the culture medium and therefore does not add any technical feature characterizing the culture medium itself. In this context, it is noted that claim 5 refers to non human embryonic stem cells. Embryonic stem cells cannot be adult mammalian cells. Therefore the subject matter of claim 5 contradicts with new claim 1 (adult mammalian cells). The same objection applies to claim 25.

The subject matter of claim 11 relates to a culture medium according to claims 4-6,

which culture medium is the same as that according to claims 1-3. This is because claims 4-6 only relate to an intended use, not to the use itself (claims 4-6 are product claims). Claim 11 refers to the culture with mammalian cells in order to further condition the MMH-conditioned medium. This is also considered as an intended use and therefore does not constitute a technical feature characterizing the culture medium. Thus it follows that the culture medium according to claim 11 still has the same technical features as that of claims 1-3.

Claim 12 relates to a process for the production of a culture medium. This process comprises two steps: (1) incubating immortalized untransformed hepatocytes ... in a culture medium and (2) separating said hepatocytes from the medium (at the indicated time point: before using the medium for culturing other cells). Claim 12 does not include the step of using the medium for culturing cells.

Claim 30 relates to a kit comprising the culture medium according to claims 1-11 together with instructions for use. This claim is a product claim. The fact that the kit (the culture medium) is suitable for a certain application (maintenance of adult mammalian cells) is not considered a technical feature characterizing the kit (=product) itself.

- 1.3 Document D2 discloses the use of murine MMH cells as feeder cells and the co-culture of these MMH cells with fetal liver hematopoietic cells (progenitors). D2 also mentions on p.1653 (column 1, paragraph 2) that fetal hematopoietic progenitor cells were cultured in MMH-conditioned medium (based on Iscove's medium, see p. 1646, column 1, §3). The MMH cells grow in monolayers and require collagen I for attachment (p. 1647, column 2., §2). Said culture results in differentiation of progenitor cells but not in the maintenance of progenitor cells. Thus by disclosing culture in MMH-conditioned medium, D2 inherently discloses culture medium conditioned by MMH cells. MMH cells are inherently genetically modified due to the method by which they have been provided (see document D3 and D4). Therefore D2 anticipates the subject matter of claims 1-9 and 11-12, 14-16.
- 1.4 The subject matter of claims 17-22 relates to mammalian cells treated with the conditioned culture medium, or pharmaceutical compositions comprising said

mammalian cells. Said mammalian cells, defined as a product by process, do not appear to differ from any known mammalian cells cultured in any other media, or from mammalian cells co-cultured with MMH cells as disclosed in D2 and subsequently purified. Therefore document D2, as well as any adult mammalian cell known in the art, anticipates the subject matter of claims 17-22.

- 1.5 Claims 10 and 13 do not appear to include any additional matter that could render them inventive as such. Claim 30 also does not involve an inventive step: no inventive activity would be required to provide a kit containing the culture medium as disclosed in D2.
- 1.6 The subject matter of claims 23-29 relates to the **use** of the conditioned medium according to claims 1-11 for growing, expanding, maintaining and differentiating isolated adult mammalian cells in vitro. The applicant has argued in the letter dated 12.07.05 that document D2 only discloses the culturing of fetal liver hematopoietic cells. To render the claimed subject matter novel over D2 the applicant has restricted the claims to the culturing of adult mammalian cells. The ED, however, considers that D2 also refers to adult cells: on p. 1647 (column 2, § 3) D2 discloses that similar results were obtained with bone-marrow derived hematopoietic cells. It is noted that the examples of the present application have also been performed with bone marrow derived hematopoietic cells. In addition D2 discloses on p. 1652 (column 2, § 1) that all MMH lines supported hematopoiesis from fetal liver or bone marrow hematopoietic cell progenitors and precursors. D2 thus also discloses the coculture of MMH cells with bone-marrow derived hematopoietic cells, which thus includes adult mammalian cells.

It is not directly evident from the passage on p. 1653 (§ 2) relating to experiments with the microporous transwell insert and with the MMH-conditioned medium, whether the latter experiments were also performed with bone-marrow derived cells. But even if this would not be the case, then the disclosure of D2 (relating to successful coculture of MMH cells with bone marrow derived hematopoietic cells) would render the use of the MMH-conditioned medium to the culture of adult cells not inventive. In other words: the disclosure that adult cells can be co-cultured with MMH cells would render it obvious that adult cells can also be cultured in MMH-conditioned medium, in analogy to the results with fetal cells cultured in MMH-conditioned medium. Therefore

no inventive step can be recognized for the use of the MMH-conditioned medium for the culture of adult cells.

- 1.7 In addition, it is noted that D2 discloses on p. 1653 (§ 2) that for long-term maintenance (in contrast to differentiation) of hematopoietic progenitor cells a **direct contact** between MMH cells and hematopoietic progenitor cells is required. It is further noted that the present application does not provide any experimental evidence for long-term maintenance of hematopoietic cells in MMH-conditioned medium (all examples relate only to expansion and differentiation). Therefore the ED considers that the present invention does not solve the problem with respect to the embodiment of 'maintenance'.

2. Industrial applicability (Article 33(4) PCT)

The subject matter of claims 1-30 appears to be industrially applicable.